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Chaetomium siamense sp. nov., a soil isolate from Thailand, produces a new chaetoviridin, G

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Abstract — *Chaetomium siamense* sp. nov., isolated from soil in a pineapple plantation in Thailand, is described and illustrated. The new species is similar to *C. cupreum* in having superficial globose or ovate ascomata with red circinate ascomatal hairs but can be distinguished by its fusiform ascospores with two apical germ pores. Phylogenetic analysis of ITS rDNA sequence data also supports *C. siamense* as distinct. Investigation of the secondary metabolites of *C. siamense* led to the isolation of one new (chaetoviridin G) and seven known compounds (ergosterol, 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol, ergosterylplamitate, cochliodone D, chaetoviridin A, chaetoviridin F, chrysophanol).

Key words — *Ascomycota, Chaetomiaceae,* novel compound

Introduction

Chaetomium Kunze, established in 1817, is an ascomycete genus in the family *Chaetomiaceae* (von Arx et al. 1986). *Chaetomium* is one of the largest genera of saprotrophic ascomycetes, comprising more than 300 species worldwide (Seth 1970, von Arx et al. 1986, Cano & Guarro 1987, Carris & Glawe 1987, Moustafa & Ess El-Din 1989, Horie & Udagawa 1990, Abdullah & Zora 1993, Udagawa et

al. 1994, Gené & Guarro 1996, Decock & Hennebert 1997, Udagawa et al. 1997, Rodríguez et al. 2002, Wang & Zheng 2005a,b). *Chaetomium* is characterized by superficial ascomata, usually covered with hairs or setae, surrounding a rather wide ostiolar pore at the apex. The ascospores are aseptate, brown or gray-olivaceous, and with one or two germ pores (von Arx et al. 1986).

During a study on antagonistic fungi for controlling root rot disease of pineapple, numerous strains of *Chaetomium* were isolated from soil using a baiting technique (Soytong & Quimio 1989). Although several species have been only recently recorded for Thailand (Pornsuriya et al. 2008), one isolate could not be identified as any known *Chaetomium* species. This isolate is here described and illustrated as *Chaetomium siamense* sp. nov., and is compared with *C. cupreum*, the most similar species. Molecular evidence, also provided, supports this species as an independent taxon.

Materials & methods

Sampling, isolation and morphological study

Soil samples were collected from pineapple plantations in Phatthalung Province, Thailand, during August to November 2007. They were placed in clean plastic bags for transport to the laboratory at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. *Chaetomium* species were originally isolated by the baiting technique described by Soytong & Quimio (1989). *Chaetomium* species developing on baits were observed daily and their ascomata transferred to glass slides with a small amount of sterilized water before being spread on water agar (WA) in a 9-cm diameter Petri dish and then incubated for 24 h at room temperature, Individual colonies were transferred onto potato dextrose agar (PDA) and corn meal agar (CMA) for identification. Morphological structures of the new species were observed under compound and scanning electron microscopes. The holotype is deposited in Biocontrol Research Unit and Mycology Section (KMITL).

DNA extraction, polymerase chain reaction (PCR) and data analysis

Genomic DNA was extracted from a mycelial culture grown in potato dextrose broth (PDB) by using Dneasy Plant Mini kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS1, 5.8S and ITS2 regions) was performed with primer pair PN3 and PN16 (Neuvéglise et al. 1994). The reaction was carried out in 25 µl volumes containing 1 µl genomic DNA, 0.5 µl dNTPs, 1 µl of each primer and 0.2 µl Taq DNA polymerase in 2.5 µl PCR buffer. Amplifications were performed with an initial denaturation step of 95°C for 5 min followed by 35 cycles of 94°C for 1 min, 60°C for 2 min and 72°C for 3 min and a final extension of 72°C for 5 min. The PCR product was separated on an agarose gel and purified for DNA sequencing by the U-gene Gel Extraction Kit II (U-gene Biotechnology Co, P.R China). DNA sequences of related species, based on the ITS including 5.8S gene data, were retrieved from GenBank (Table 1). *Chaetomium nigricolor* and *Chaetomium* sp. 15004 were used as outgroup. All sequences were assembled using BioEdit ver. 7.0.2 and aligned using ClustalX ver 1.83. Phylogenetic relationships were determined by

Table 1. *Chaetomium* species and isolates used in this study

isolates	ITS GenBank accession No.	host/substrate	geographic origin
C. atrobrunneum	FJ595483	Chinese child	China
C. bostrychodes	EU520130	—	China
C. cupreum	AB509366	soil	Thailand
	AB509367	soil	Thailand
	AB509368	soil	Thailand
	AB509369	soil	Thailand
	AB509370	soil	Thailand
	AB509371	soil	Thailand
	AB509373	soil	Thailand
C. funicola	EU552109	*Leucadendron* sp.	South Africa
	EU139246	*Macrotermitinae* nest	France
C. globosum	FJ538594	chrysanthemum	USA
C. hispanicum	DQ069021	*Picea abies*	Lithuania
C. longicolleum	GQ922525	—	Netherlands
C. murorum	GQ376100	deteriorated painting	Greece
C. nigricolor	EU543258	*Dioscorea zingiberensis*	China
C. pachypodioides	GQ922526	—	Netherlands
Chaetomium sp. 15004	EU750692	—	China
Chaetomium sp. aurim1182	DQ093660	*Picea abies*	Lithuania
Chaetomium sp. INBI2-26(-)	AJ620951	soil	Vietnam
C. thermophilum	EF550983	—	India

heuristic search under neighbor joining (NJ) using PAUP* 4.0b8 (Swofford 2001). Tree was drawn using Treeview.

Identification of metabolites

Erlenmeyer flasks (1 L) containing 200 ml PDB were inoculated with mycelial plugs from a 5-day-old culture of *C. siamense*. The flasks were incubated for 4 weeks on stationary state at 27–30°C. Mycelium mats were then harvested, air dried, ground, and extracted with a series of methanol, ethyl acetate and hexane. The crude extract was loaded onto a silica gel column. The column was successively eluted to obtain different fractions and monitored by TLC, and reduced to one active fraction. The chemical structures isolated from dried mycelial mats of *C. siamense* were elucidated on the basis of spectroscopic evidence (IR, ^{1}H NMR, and ^{13}C NMR and 2D-NMR).

Taxonomy & discussion

Chaetomium siamense Pornsuriya & Soytong, **sp. nov.** Figs 1

MycoBank MB 506801

Coloniae modica bardus crescents in agaris PDA et CMA, cum mycelio aerio; reversum rubus. Mycelium ex hyphis hyalinis vel subhyalinis, septatis, laevibus, 1.8–2.3 µm diam compositum. Ascomata superficialia, globosae, ovata, ostiolata, fuscobrunneum in refulgens, paramariae ad 87–148 × 107–177 µm. Peridium fuscobrunneum et ex textura angularis; epidermoidea ex fuscus, paramariae ad 2.8–5.7 × 5.1–10.4 µm. Ascomatal pili abundantibus, simplici, aurantiacus in refulgens, spira in ultima parte et ex septati,

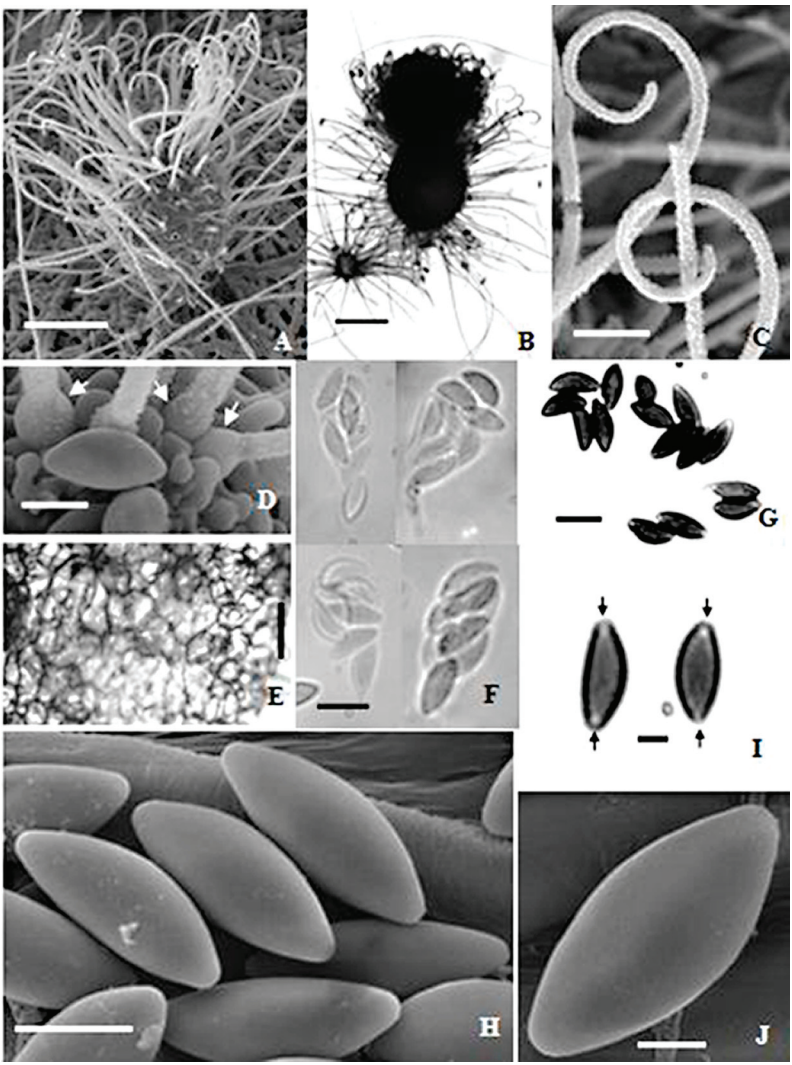

Figure 1. *Chaetomium siamense*. A. Ascoma viewed under a scanning electron microscope. B. Ascoma viewed under a light microscope. C–D. Arcuate ascomatal hairs with swollen basal cells (arrowed) viewed under a scanning electron microscope. E. Peridium viewed under a light microscope. F. Asci viewed under a light microscope. G. Ascospores viewed under a light microscope. H. Ascospores viewed under a scanning electron microscope. I. Fusiform ascospores with two apical germ pores (arrowed). J. Ascospore viewed under a scanning electron microscope. Bars: A, B = 50 µm; C, E–G = 10 µm; D, H = 5 µm; I, J= 2 µm.

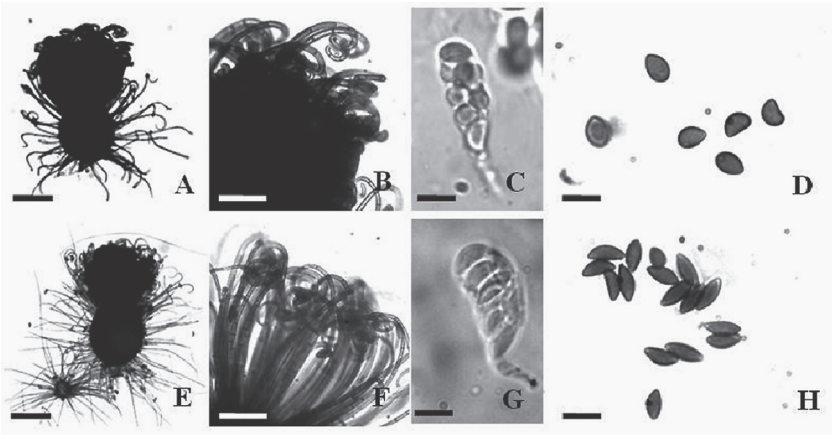

Figure 2. Comparison of the characteristics of *Chaetomium cupreum* (A–D) and *C. siamense* (E–H). Bars: A, E = 50 µm; B–D, F–H = 10 µm.

1.3–4 µm diam, 170 µm longi. Pili laterals recti, leviter, verrucosi, paramariae ad 56–160 µm longi. Asci numerosis, fasciculati, clavati, pedicellati, 8-spori, 9–14 × 28–40 µm. Ascosporae fusiformia, fascobrunneum, laevibus, 4.3–5 × 11.1–13.5 µm, cum 2 poro germinalibus apicali visibile.

Holotype: Thailand, Phatthalung, isolated from soil in a pineapple plantation, 27 September 2007, Chaninun Pornsuriya TMACC001, deposited in herbarium of the Biocontrol Research Unit and Mycology Section, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand.

Etymology: *siamense* refers to Thailand (Siam), country of origin.

Colonies on PDA and CMA growing moderately slowly, covering 9 cm Petri dish surface after 14 days at room temperature, with aerial mycelium; becoming red due to red pigment exudates when young, yellow when old and formation of ascomata. Mycelium composed of hyaline to subhyaline, septate, smooth hyphae, 1.8–2.3 µm diam. Ascomata discrete, maturing within 10–14 days, superficial, globose or ovate, ostiolate, dark brown in reflected light, 87–148 × 107–177 µm. Peridium dark brown, of textura angularis; outer layer composed of brown, thick-walled cells measuring 2.8–5.7 × 5.1–10.4 µm. Ascomatal hairs abundant, unbranched, red or orange-red in reflected light, apically circinate or coiled, regularly septate and forming a dense tuft around the ostiole, finely verrucose towards the tip, up to 170 µm long, 1.3–4 µm diam near the base. Lateral hairs less abundant, straight, finely verrucose, 56–160 µm long. Asci numerous, fasciculate, clavate, stalked, 8-spored, 9–14 × 28–40 µm. Ascospores fusiform, dark brown at maturity smooth and rather thick-walled, 4.3–5 × 11.1–13.5 µm, with two apical germ pores.

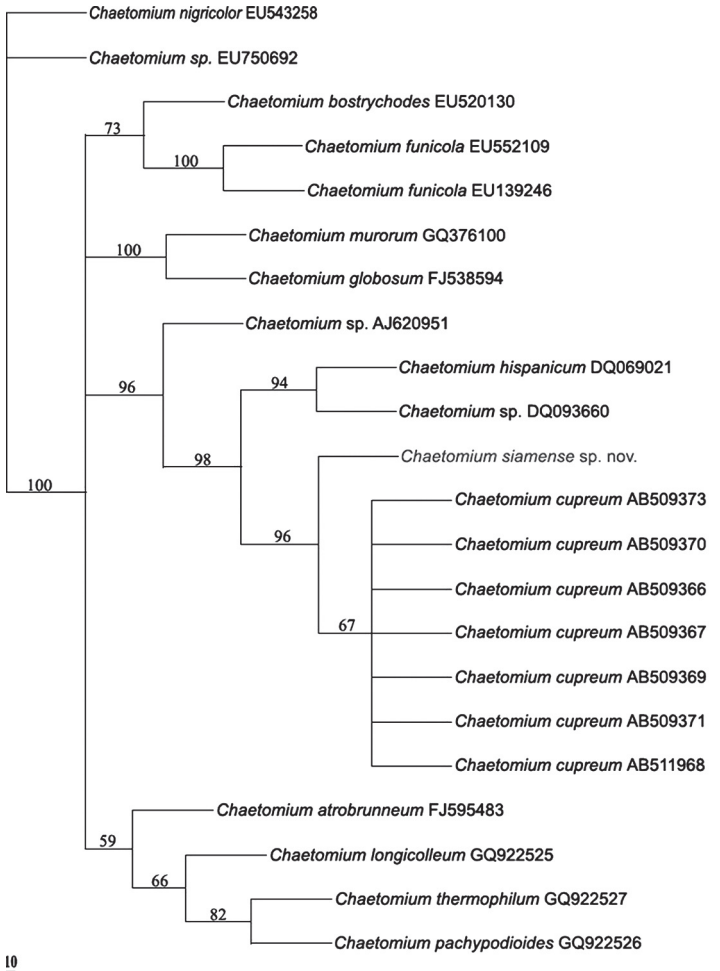

Figure 3. Phylogenetic tree of *Chaetomium* spp. from GenBank including *Chaetomium siamense* constructed after distance-based analysis of ITS1 and 5.8S regions of rDNA. Numbers at the branches indicate the percentage of bootstrap values after 1000 replications. The outgroup taxa are *Chaetomium nigricolor* and *Chaetomium* sp.

Comments: *Chaetomium* is a large ascomycete genus with species inhabiting various cellulose-based substrates including paper and plant compost. After review of the genus by von Arx et al. (1986) several new species have been described: *C. biporatum* (Cano & Guarro 1987), *C. histoplasmoides* (Carris & Glawe 1987), *C. sinaiense* (Moustafa & Ess El-Din 1989), *C. subcurvisporum*

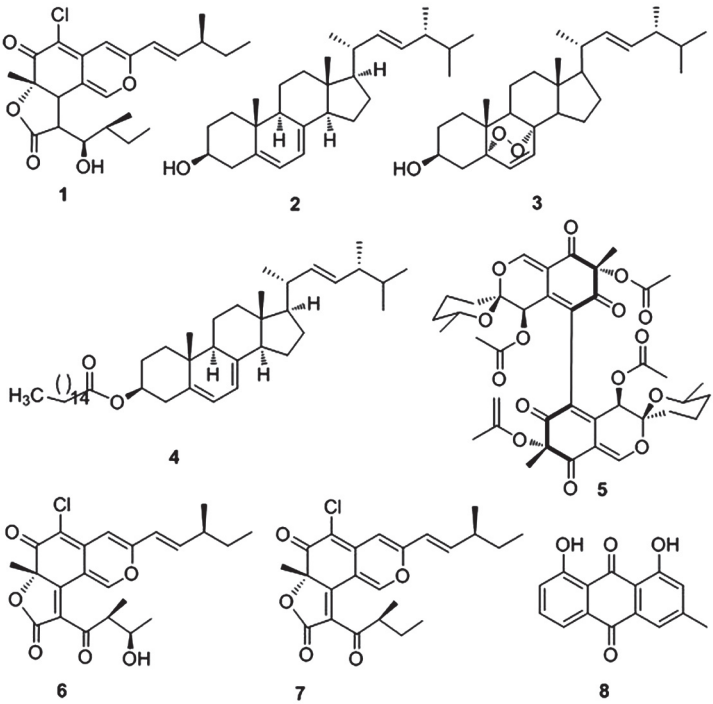

Figure 4. Compounds isolated from *Chaetomium siamense*. 1. chaetoviridin G; 2. ergosterol; 3. 24(R)-5α,8α-epidioxyergosta-6-22-diene-3β-ol; 4. ergosterylplamitate; 5. cochliodone D; 6. chaetoviridin A; 7. chaetoviridin F; 8. chrysophanol.

(Abdullah & Al-Bader 1989), *C. myricicola* (Horie & Udagawa 1990), *C. mesopotamicum* (Abdullah & Zora 1993), *C. novae-caledoniae* (Udagawa et al. 1994), *C. floriforme* (Gené & Guarro 1996), *C. cuyabenoensis* (Decock & Hennebert 1997), *C. umbratile* (Udagawa et al. 1997), *C. macrostiolatum*, *C. olivicolor*, *C. tarraconense* (Rodríguez et al. 2002) *C. acropullum* (Wang & Zheng 2005a), and *C. ampulliellum* (Wang & Zheng 2005b). *Chaetomium siamense* can be distinguished from other species by its superficial, spherical or ovate ascomata with angular brown walls. Ascomatal hairs are red or orange-red in reflected light, arcuate, apically circinate or coiled, septate and ascospores are fusiform with two apical germ pores. The new species was initially identified as *C. cupreum* L.M. Ames, which produces red pigment in agar medium and which also produces superficial spherical or ovate ascomata (Figs. 2A,E) with a peridium of angular brown-walled cells.

Ascomatal hairs are red, arcuate, apically circinate or coiled and septate (Figs. 2B,F). However, ascospore morphology easily separates the new species

from *C. cupreum* (Figs. 2H,D). The longer (11.1–13.5 µm) fusiform ascospores with two apical germ pores (Fig. 2H) of *C. siamense* clearly differ from the shorter (7–10 µm) reniform ascospores with a single apical germ pore (Fig. 2D) that characterize *C. cupreum*. Although the phylogenetic analysis indicates that *C. siamense* is related to *C. cupreum,* sequence differences (as shown by the separate branch tips, Fig. 3) clearly support *C. siamense* as a distinct species.

Investigation of the secondary metabolites from crude extracts of dried mycelial mats of *C. siamense* led to the isolation of a new compound named chaetoviridin G (1). Seven other known compounds — ergosterol (2), 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (3), ergosterylplamitate (4), cochliodone D (5), chaetoviridin A (6), chaetoviridin F (7), and chrysophanol (8) — were also identified. Structures were elucidated based on spectroscopic evidence (IR, ^{1}H NMR, and ^{13}C NMR and 2D-NMR). Compounds 2–8 (Fig. 4) have also been reported from *C. cochliodes* (Phonkerd et al. 2008).

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